The accumulation of SET protein in Head and neck squamous cell carcinomas (HNSCCs) increases basal autophagy and prevents mitochondrial dysfunction

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Introduction: We previously demonstrated that SET accumulation in head and neck squamous cell carcinomas (HNSCCs) promotes cell survival under oxidative stress in association with p-Akt up-regulation and that SET may act as either a cell survival signal or an oxidative stress sensor. When overexpressed in HEK293T cells, SET enhanced autophagic flux and protected mitochondria under oxidative stress. Here, we performed SET knockdown (siSET vs. siCN) in two HNSCC cell lineages and treated cells with the pro-oxidant t-butyl hydroperoxide (t-BHP, 250 and 500 µM): HN13, that accumulates SET and is resistant to death, and CAL27, as common HNSCC used in cancer research.

Methods: SET, Akt, LC3B and p62 protein levels were analyzed by immunoblotting; mitochondrial membrane potential was estimated with JC-1 fluorescent probe; intracellular reactive oxygen species (ROS) were monitored by oxidation of fluorescein; cell viability was estimated by calcein retention; autophagy flux was assessed by LC3B cleavage, and formation of acidic vacuolar organelles (AVOs) by immunofluorescence.

Results and Discussion:
(1) Oxidative stress decreased both SET and total Akt levels in CAL27, but not in HN13 cells, regardless the presence of SET knockdown; (2) the viability of HN13 cells was reduced with SET knockdown only after treatment (t-BHP, 500 µM) and CAL27 cells were more sensitive to SET knockdown, with loss of viability even in the absence of oxidative stress; (3) while mitochondrial membrane potential of CAL27 cells was sensitive to oxidative stress, independent of SET, in HN13 cells it was depressed only after SET knockdown; (4) CAL27 cells showed higher ROS accumulation in the presence of oxidative stress with SET knockdown; (5) HN13 cells showed a SET-dependent basal increased autophagic flux, estimated by LC3B cleavage, as well as formation of acidic vesicular organelles (AVOs); (6) CAL27 cells underwent apoptosis after oxidative stress, as demonstrated by PARP cleavage and caspase-3 activation.

Conclusions: These findings support a role of SET accumulation in maintaining mitochondrial energetics and modulating autophagy in HNSCCs.

Keywords: oxidative stress, cancer, SET, autophagy

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